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THE vascular architecture of the brain of C57Black/6 and SV129 mice was studied following microvascular injection of carbon black stained latex. The dorsal brain surface was photographed to determine the number, diameter, and position of pial anastomotic vessels between the middle and anterior cerebral arteries. The mean number and diameter of anastomoses were not significantly different, but the line of anastomoses interconnecting the half way points of anastomotic vessels was located significantly closer to the midline in C57Black/6 mice, demonstrating that the middle cerebral artery had a larger vascular supplying territory than in SV129 mice. This explains the larger infarct volume previously reported in C57Black/6 mice, and raises concerns about the use of C57Black/6 and SV129 mice as parent strains for genetically modified animals in stroke research. *NeuroReport* 9: 1317-1319 © 1998 Rapid Science Ltd.

Key words: Anastomoses; Cerebral arteries; Cerebral vasculature; Mice; Mutant; Strain difference

Differences in the cerebrovascular anatomy of C57Black/6 and SV129 mice

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Introduction

In recent years, genetically engineered animals have been used increasingly to investigate the molecular mechanisms of ischemic brain injury.¹⁻³ The most widely used parent strains for the production of mutants are C57Black/6 and SV129 mice, even though brain infarcts after permanent middle cerebral artery (MCA) occlusion are larger in the C57Black/6 than in the SV129 strain.⁴ This finding is disturbing, because differences in infarct volume after targeted gene mutation could be confused with differences in the genetic background of the two parent strains.

The most likely reason for strain differences in infarct volume is a difference in the vascular anatomy. The supplying territories of the major cerebral arteries are interconnected by Heubner's pial anastomoses which determine the efficiency of the collateral blood supply. Differences in the number and in the size of such anastomoses will, therefore, result in differences in the severity of the ischemic impact. Another anatomical factor could be a difference in the size of the vascular territory. In fact, the angioarchitecture is not strictly related to the gross anatomy of the brain and, therefore, may vary in different strains.

A straightforward way of investigating both the size of the vascular territory and the number and diameter of the anastomoses interconnecting these territories is the injection of the vascular system with

latex, as proposed by Coyle and Jokelainen.⁵ The resulting staining of the pial vessels facilitates the visualization of the peripheral branches of the supplying brain arteries and allows the precise identification and localization of the anastomotic vessels connecting these branches. Here, we demonstrate that the angioarchitecture of MCA does, in fact, differ in C57Black/6 and SV129 mice.

Materials and Methods

Experiments were carried out according to the NIH guidelines for the care and use of laboratory animals, and approved by the local authorities. Six male C57Black/6 mice and six male SV129 mice aged between 10 and 12 weeks were used (Harlan Winkelmann, Borcheln, Germany). Animals were housed under diurnal lighting conditions and allowed access to food and water *ad lib* before the experiment. Anesthesia was induced by 1.5% halothane and maintained with 1% halothane in 70% N₂O and 30% O₂. Rectal temperature was maintained at 36.5-37.0°C throughout the experiment, using an infrared lamp and a heating pad for feedback-controlled temperature stabilization (YSI, Yellow Springs, OH, USA).

The procedure used for visualization of the brain vasculature in mice was basically that described by Coyle and Jokelainen in rats.⁵ A lethal dose of papaverine hydrochloride (40-50 mg/kg, i.v. in sterile water) was injected to produce maximal vasodilation

and to minimize cerebrovascular resistance. The thoracic aorta was clipped at the level of diaphragm and cannulated with polyethylene tubing (internal diameter 0.58 mm, Portex, England). Warm (38°C), undiluted Vultex (a white latex; Chicago Latex Products no. 563) was mixed with a small amount of carbon black (10 µl/g, Bokusai; Fueki, Tokyo, Japan) and was injected into the ascending aorta. The injection volume of latex was 0.4 ml, as determined in preliminary trials; the injection pressure was about 150 mmHg.

Thirty minutes after the injection, the animal was decapitated and the dorsal part of the skull and the dura were removed. To prevent deformation of the brain, the entire head was fixed in 10% formalin for 4 weeks before brain removal. Thereafter, the dorsal side of the brain was inspected under the operating microscope and photographed at $\times 20$ magnification. Preparations with unstained or ruptured pial vessels were excluded from the study.

Anastomoses on the dorsal surface of the hemispheres were localized by tracing the peripheral branches of the anterior cerebral artery (ACA) and the MCA to the anastomosis point, defined as the narrowest part of the vessel or half way between the nearest branching points of the ACA and the MCA branches, respectively.⁵ Adjacent anastomosis points were connected by the line of anastomoses,⁶ and the distance from the midline to the line of anastomoses was measured on photographs taken from the dorsal brain surface at coronal planes 2 mm, 4 mm and 6 mm from the frontal pole. The number of the anastomoses per hemisphere was counted, and the diameters of the two largest anastomoses in each hemisphere were measured using an image analyzer and NIH image 1.59 software (National Institutes of Health, Bethesda, MD, USA).

All values are given as means \pm s.d. The interstrain difference in the distance of the line of anastomoses from the midline was tested by one-way analysis of variance followed by Scheffé's *post hoc* analysis. Differences in the number and diameter of anastomoses between strains were tested for statistical significance using Mann-Whitney's U test.

Results

Figure 1 shows the dorsal aspect of the pial vasculature of a C57Black/6 mouse (left) and a SV129 mouse (right). In C57Black/6 mice the line of anastomoses between the ACA and the MCA was clearly closer to the midline than in SV129 mice. Quantitative evaluation of the distance between the line of anastomoses and the midline at three levels – 2 mm, 4 mm and 6 mm from the frontal pole –

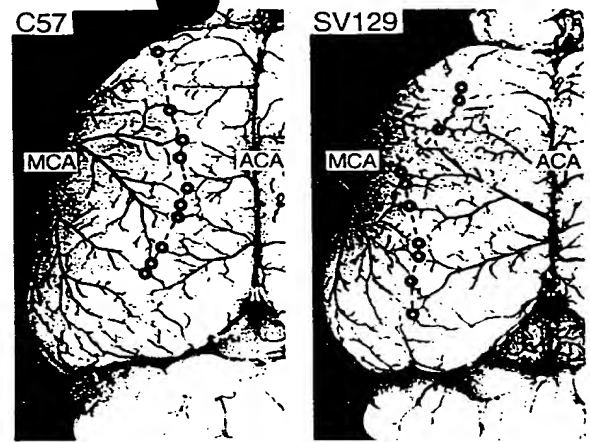


FIG. 1. Dorsal view of the cerebral hemisphere of C57Black/6 mice (left) and SV129 mice (right) after microvascular injection with carbon black stained latex. The points of anastomoses between the middle cerebral artery (MCA) and anterior cerebral artery (ACA) are marked with circles and connected by the line of anastomoses. Note the marked shift of the line of anastomoses to the midline in C57Black/6 mice.

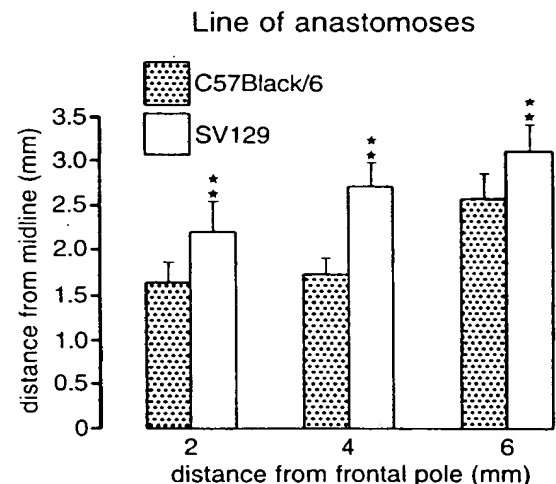


FIG. 2. Distance of the line of anastomoses from the midline in C57Black/6 mice (dotted bars) and SV129 mice (white bars) in three coronal planes, located 2 mm, 4 mm and 6 mm from the frontal pole. Note significantly smaller distance in the C57Black/6 mice, reflecting the larger MCA supplying territory in this strain (** $p < 0.01$).

revealed significant differences ($p < 0.01$) of up to 1 mm (Fig. 2).

In contrast to the position of the line of anastomoses, the number of anastomoses did not differ significantly in C57Black/6 mice (9.50 ± 1.51 per hemisphere) and SV129 mice (11.1 ± 2.50 per hemisphere). The diameter of anastomoses did not vary, either (C57Black/6: 26.6 ± 7.4 µm; SV129: 23.4 ± 5.7 µm).

Discussion

The architecture of the cerebral vascular system has been visualized by filling the microcirculation with low viscosity resin,⁷ Araldite F,⁸ gelatin mixed with India ink,⁹ or latex.⁵ Coyle and Jokelainen, who introduced the latex infusion method in the rat, infused a high dose of papaverine in order to produce maximal vasodilation and to minimize vascular resistance.⁵ We confirmed that this procedure also produced consistent cerebrovascular filling in mice but we found it difficult to detect small vascular branches because the white colour of latex produced too little contrast to the colour of the formalin-fixed brain tissue. We therefore modified the technique by staining the latex with carbon black. This modification allows easy demarcation even of the finest arterial anastomoses, and also facilitates the measurement of vascular diameter.

Using this method, marked differences of angioarchitecture were detected between C57Black/6 and SV129 mice. The supplying territory of the MCA was significantly larger in C57Black/6 mice, which readily explains the larger infarct volume observed in this strain.⁴ However, the volume of infarct also depends on other hemodynamic and molecular variables. Coyle and his co-workers reported that the diameter of anastomoses is an important factor in determining the supply to the occluded vascular territory.^{6,10,11} Our measurements, however, revealed that both the number and the diameter of papaverine-dilated anastomoses were identical in the two strains. Under ischemic conditions, C57Black/6 mice may even have an inherent hemodynamic advantage over the SV129 strain because the vasodilatory response to acetylcholine is more robust.¹² Another advantage of C57Black/6 mice could be their lower susceptibility to excitotoxicity, as observed after kainic acid-induced seizures.¹³ However, the investigation of hippocampal injury after global ischemia which is thought to result from excitotoxicity¹⁴ revealed just the opposite.¹² Thirty minutes bilateral carotid occlusion in C57Black/6 mice caused the same degree of cellular damage as 75 min of ischemia in SV129 mice, probably due to a hypoplastic posterior communicating artery in the former strain.¹² This example demonstrates that hemodynamic and molecular mechanisms of ischemic injury are intricately intermingled, and that it is impossible to predict ischemic susceptibility by consideration of any pathogenetic factors alone. The larger vascular territory supplied by the MCA in C57Black/6 mice is, therefore, a likely factor but not necessarily the only one responsible for the larger infarct volume after occlusion of this artery in this strain.⁴

The present demonstration of the angioarchitectural differences in C57Black/6 and SV129 strains adds to the growing concern over the use of genetically engineered mice for the study of ischemic injury. In most of these investigations, embryonic stem cells are derived from SV129 mice, and after gene targeting the resultant chimeric mice are mated with C57Black/6 mice.¹⁵ The offspring, therefore, carry both C57Black/6 and SV129 genes. According to the theory of genetic linkage, pseudo-correlations may evolve if the targeted gene locus is located close to the gene related to cerebral arterial architecture. It is, therefore, mandatory to confirm that independent variables, such as differences in the vascular architecture, do not interfere with the result of gene targeting. An alternative approach would be backcrossing with a single parent strain for at least 12 generations to ensure homogeneity of genetic background.¹⁵ However, as pointed out by Fujii *et al.*,¹² such an approach is time-consuming and expensive. Visualization of the vascular supplying territory is a much simpler and more straightforward method of detecting or refuting the existence of such interactions, and is strongly recommended.

Conclusions

The supplying territory of the middle cerebral artery is significantly larger in C57Black/6 mice than in the SV129 strain. This explains the previously documented difference in infarct volume in C57Black/6 and SV129 mice and adds to the growing concern about the use of these animals as parent strains for genetically engineered mutants in stroke research.

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